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14. ABSTRACT

We are testing the hypothesis that differential telomere loss underlies the elevated risk of prostate cancer observed in African Americans and in families displaying a genetic cancer predisposition. To test this hypothesis, telomere content in genomic DNA isolated from blood samples of relevant cohorts will be determined and assessed for associations with either racial background or prostate cancer in members of prostate cancer families. Over the period of this report we have isolated genomic DNA from 128 members from prostate cancer families lacking strong genetic linkage between cancer risk and specific genomic loci. Buffy coat has been isolated from anonymous cord blood samples collected from newborns of different racial backgrounds and DNA is currently being isolated form these samples. We are currently in the queue to receive mid-life buffy coat samples from the Health Professionals Follow-up Study. The primary method for determining telomere content in genomic DNA samples, a quantitative real-time PCR assay, has now been established in our lab.

15. SUBJECT TERMS
Telomere, Prostate Cancer, Health disparity, Familial prostate cancer, Carcinogenesis

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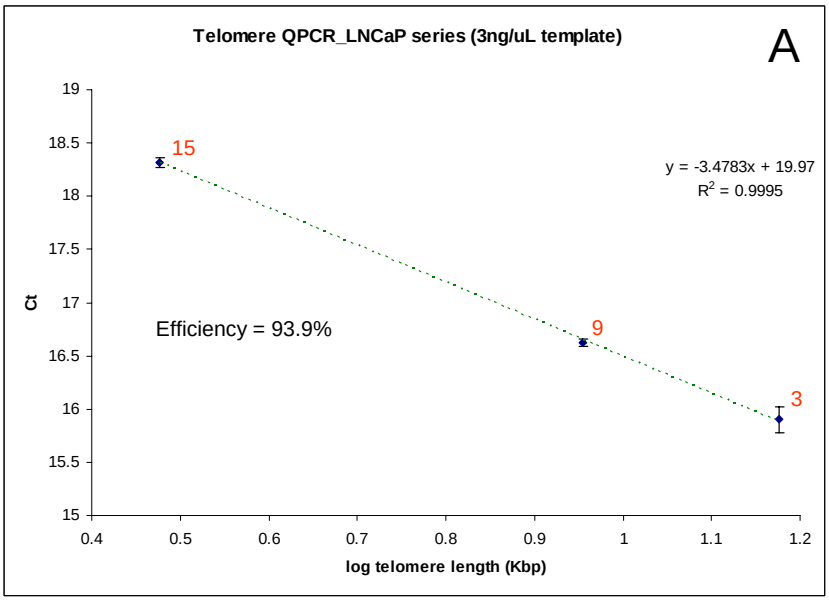
Introduction

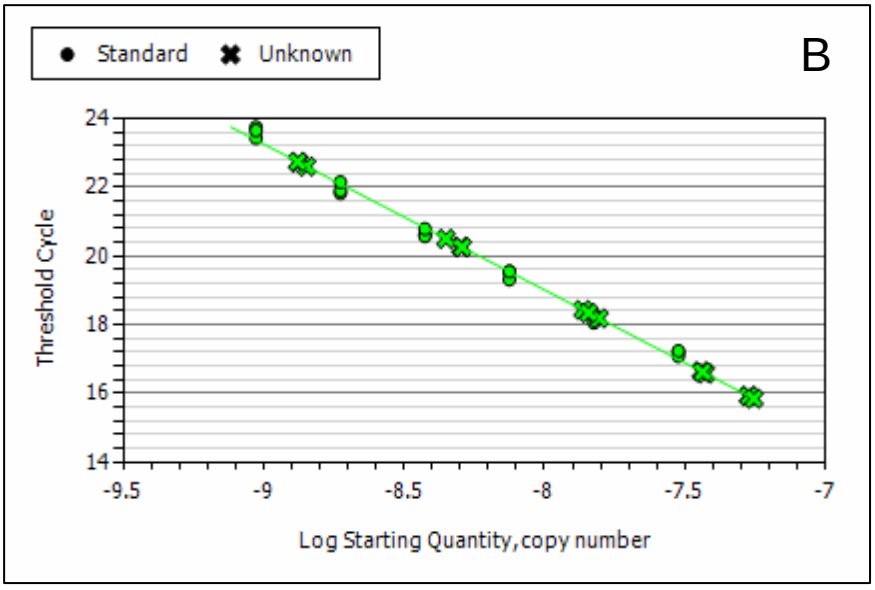
The overall goal of our research is to determine the role played by telomere biology in human prostate cancer. In this proposal we are testing the hypothesis that telomere length abnormalities play a causal role in prostate carcinogenesis. Accordingly, we are testing this in two populations enriched for prostate cancer - African American men, and male members of prostate cancer families. If the hypothesis is correct, we expect to find (i) significantly shorter telomeres in African American males versus age-matched Caucasian males and (ii) an association between shorter telomeres in affected members of prostate cancer families versus age-matched unaffected relatives. Such support for the hypothesis would highlight the importance of telomere biology in prostate cancer, thus future studies in this area could lead to new targets for the prevention and treatment of prostate cancer. Furthermore, a defined link between telomere length, as measured in peripheral blood samples, and prostate cancer risk could lead to a simple blood test for assessing a man's risk of developing prostate cancer.

Body

Summary of timeline: This PCRP New Investigator Award had a December 2005 start date, with the included provision that approval for the use of human subjects was still pending at that time. Final written authorization for the use of human subjects material was received one year later, thus there was a one year delay in initiating the research as it utilizes human-derived blood and DNA samples. Therefore, during the previous reporting period (Year 1) we procured supplies and established general quantitative real-time PCR (Q-PCR) methodology in the lab.

Current progress: During the reporting period covered in this annual report, we set up the Q-PCR method for quantifying telomere DNA content in our lab and validated it using DNA derived from cells with known telomere lengths, independently measured by a Southern blot assay (Figure 1A). Further assay validation was conducted using a DNA dilution series of genomic DNA isolated form cultured normal human cells as well as blood-derived DNA (Figure 1B).





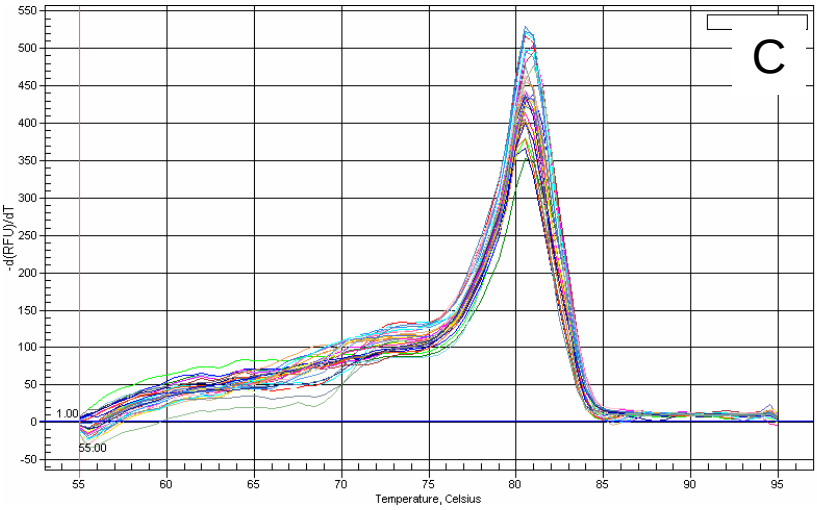


Figure 1*. Validation of Q-PCR telomere content assay*. A: Ct values from telomere DNA content Q-PCR assay (Y-axis) are strongly linearly correlated with the log of telomere length determined independently by Southern blot (x-axis). B: Standard curve (closed circles) obtained in the Q-PCR telomere content assay using serial dilutions of normal human fibroblast genomic DNA as template. C: Melt curve of products in panel B.

We encountered some trouble in implementing the telomere Q-PCR content assay and we are still conducting optimization runs in order to assure acceptable reproducibility before running the actual clinical samples. In addition, the assay requires a second Q-PCR reaction be run on each sample for normalization purposes, and we are currently determining the best genomic DNA target sequence for use for this purpose. As a backup plan, we are also developing a novel quantitative telomere PCR assay based on padlock probe technology (Figure 2). This method was presented at the 2007 DOD IMPACT Meeting in Atlanta, Georgia.

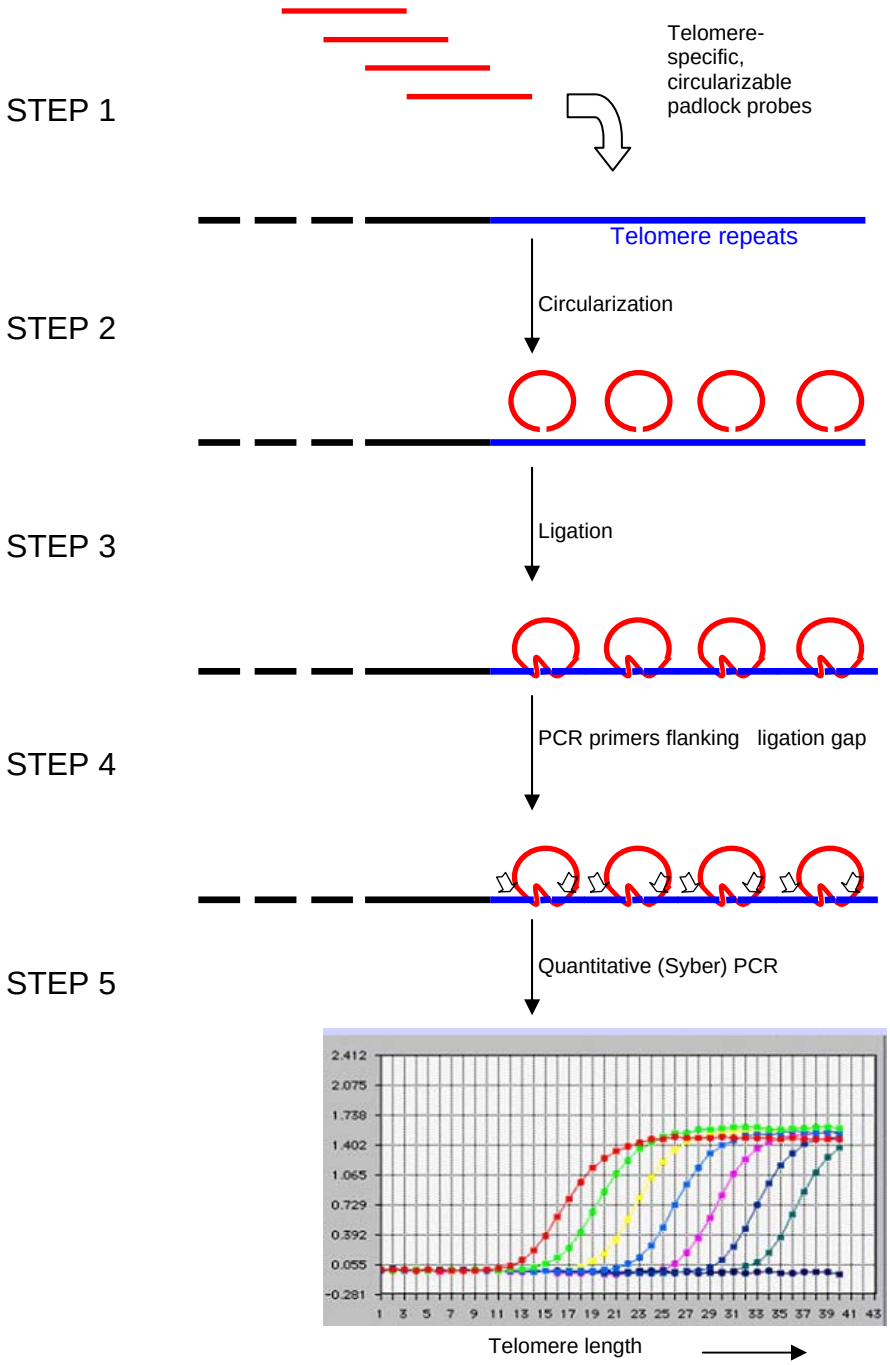


***Figure 2**. Padlock probe Q-PCR assay for telomere length assessment.* Step 1: 100-mer synthetic hybridization probes (red) anneal to genomic telomere repeat sequences (blue) via complementary sequences on the probe termini. Step 2: Hybridization results in probe circularization. Step 3: Ligation locks the circularized probes onto the genomic template. Step 4: PCR is conducted using primer sequences (white arrows) aimed across the site of ligation, such that only circularized probes will be competent for amplification. Step 5: Reaction products are quantified over time via Syber Green dye incorporation. Ct values will be inversely proportional to telomere content of the genomic DNA sample.

Sample procurement: Genomic DNA has been purified and quantified from 128 blood samples from affected and non-affected males from the Johns Hopkins Familial Prostate Cancer Registry. These samples are now ready for telomere content measurement. Buffy coat has been isolated from umbilical cord blood samples (at-birth racial variation arm) collected, anonymously; from newborns of different racial backgrounds and DNA is currently being isolated form these samples. We are currently in the queue to receive buffy coat samples from the Health Professionals Follow-up Study (mid-life racial variation arm). Once we are satisfied with the Q-PCR assay's performance and stability we will begin assaying these clinical genomic DNA samples.

Key Research Accomplishments

- Quantitative PCR (Q-PCR) telomere content assay established in lab.
- Q-PCR assay validated using control genomic DNA dilution series and genomic DNA from cell lines having known telomere contents.
- Alternative telomere content assay under development as backup.
- Genomic DNA isolated from blood samples of familial prostate cancer cohort.
- Buffy coats isolated from umbilical cord blood samples.
- Buffy coat request from Health Professionals Follow-up Study is in queue.

Reportable Outcomes

A. As a result of the support and experience received through the DOD New Investigator Award, support for additional projects related to telomere biology in prostate cancer were obtained:

Department of Defense W81XWH-05-1-0030 (Elizabeth Platz, PI)
11/01/2004-10/31/2008
Telomere Length as Predictor of Aggressive Prostate Cancer
The goals of this project are to evaluate whether telomere shortening predicts aggressive prostate cancer in cohort of men and to determine whether dietary and lifestyle factors that influence cellular proliferation or oxidative stress predict telomere length in normal appearing prostate and in peripheral blood lymphocytes.
Role: Co-Investigator

NIH/NCI P01CA108964-01A1 (Project 4; Elizabeth Platz, PI)
05/01/2005-04/30/2010
Genotypic and Phenotypic Studies of Inflammation in the PCPT
The goal of this project, which is a component of the program project entitled "Biology of the Prostate Cancer Prevention Trial (PCPT)" is to examine the contribution of the extent of intraprostatic inflammation and atrophy as assessed in biopsies, polymorphisms in genes involved in inflammation and response to infection, and presence of antibodies against infectious agents to prostate cancer.
Role: Co-Investigator

Patrick C. Walsh Prostate Cancer Research Fund
04/01/2007-03/31/2008
Specific Detection of Prostate Cancer in Urine by Multiplex Immunofluorescence and Telomere FISH – Guiding Clinical Decisions Following Negative Prostate Biopsy

The goal of this project is to develop a novel cell-based assay involving simultaneous staining of telomeres and a set of protein molecular markers to allow specific identification of prostate cancer cells in urine cytology specimens.
Role: PI

Patrick C. Walsh Prostate Cancer Research Fund
04/01/2007-03/31/2008
The Senescent Phenotype in Human Prostate Cancer: Pilot Characterization Study and Association with Aging and Cellular Stress
The goal of this project is to characterize the senescent phenotype in the human prostate, its relationship to age, modulation of the phenotype by dietary factors and oxidative damage, and how it relates to risk of prostate cancer.
Role: Co-investigator

B. Experience gained while supported by the DOD New Investigator Award contributed to the following presentations related to telomere biology:

Peer Reviewed manuscripts

1. Palapattu GS, Meeker AK, Harris T, Collector MI, Sharkis SJ, DeMarzo AM, Warlick C, Drake CG, Nelson WG. Epithelial architectural destruction is necessary for bone marrow derived cell contribution to regenerating prostate epithelium. *Journal of Urology.* 176:813-818. 2006. Cover article.

2. Hansel, D.E., Meeker, A.K., Hicks, J., De Marzo, A.M., Lillemoe, K.D., Schulick, R., Hruban, R.H., Maitra, A., Argani, P. Telomere length variation in biliary tract metaplasia, dysplasia, and carcinoma. Modern Pathology. 19:772-779, 2006.

3. Kawai T, Hiroi S, Nakanishi K, Meeker, AK. Telomere length and telomerase expression in atypical adenomatous hyperplasia and small bronchioloalveolar carcinoma of the lung. *American Journal of Clinical Pathology.* 127:254-262. 2007.

4. Stewénius Y, Jin Y, Øra I, de Kraker J, Bras J, Frigyesi A, Alumets J, Sandstedt B, Meeker AK, Gisselsson D. Defective chromosome segregation and telomere dysfunction in aggressive Wilms' tumors. *Clinical Cancer Research*. 13:6593-6602. 2007.

5. Bechan GI, Meeker AK, Marzo AM, Racke F, Jaffe R, Sugar E, Arceci RJ. Telomere length shortening in Langerhans cell histiocytosis. *British Journal of Haematology*. Published article online: 20-Dec-2007.

6. Cummings, S.D., Ryu, B., Samuels, M.A., Yu, X., Meeker, A.K., Healey, M.A., Alani, R.M. Id1 delays senescence of primary human melanocytes. *Molecular Carcinogenesis*. 2008. Epub ahead of print.

Published abstracts from presentations

1. Meeker AK, Vander Griend D, Konishi Y, Isaacs JT. Combined fluorescence in situ hybridization (FISH) for telomeres and centromeres provides rapid and simple discrimination of species of origin for cells in tissue recombination experiments. Modern Pathology21: 366A-367A 1669 Suppl. 1 Jan. 2008

2. Meeker AK, Hicks JL, Smearman E, De Marzo AM. A chromogenic in situ hybridization (CISH) technique for visualizing telomeric DNA in fixed tissue sections. Lab. Invest.86: 331A-331A 1544 Suppl. 1 Jan. 2006

3. Meeker AK, Epstein JI, Konishi Y, Netto GJ. Direct assessment of telomeres in testicular germ cell tumors reveals evidence of telomere length heterogeneity and non-telomerase mediated telomere maintenance in tumor subsets. Modern Pathology 20: 163A-163A 739 Suppl. 2 Mar. 2007.

4. Meeker AK, Bova GS, Hicks JL, De Marzo AM. Direct in situ analysis of telomere lengths in primary tumors and corresponding local and distant metastases obtained via rapid autopsy Modern Pathology 18: 155A-155A 717 Suppl. 1 Jan. 2005

5. Iwata T, Meeker AK, Smearman E, De Marzo AM. The telomere shortening in prostatic atrophy lesions Lab. Invest.88: 162A-162A 738 Suppl. 1 Jan. 2008.

Conclusion

During the current reporting period we established a previously published Q-PCR telomere content assay in our laboratory (R.M. Cawthon, 2002). Although our early validation runs indicate that the assay can function properly, we have observed some irreproducibility in running the assay with test samples, and we are currently working to correct this. In addition, as a back-up, we are developing an alternate Q-PCR method for telomeres that does not rely on PCR primer sets having significant self-complementarity, such as those used in the published protocol.

This past year was the first year in which use of human anatomical subjects was approved; therefore we began procuring blood samples and isolating genomic DNA from them for eventual assay. Specifically, DNA has been isolated from all blood samples for use in testing the hypothesis that telomere lengths are associated with prostates cancer risk in hereditary prostate cancer. Buffy coats have been isolated from umbilical cord blood samples which will be used to test for racial variation at birth in inherited telomere lengths. These samples are currently being processed for genomic DNA isolation. For testing racial variation in telomere lengths at mid-life, we will be using blood samples collected by the Health Professionals Follow-up study, and the request for these samples is currently in queue. Since we were delayed in initiating the human subjects research, we sought and were granted a one year no cost extension for this NIA award.

Support for the PI by the DOD New Investigator Award was critical in enabling a successful early career transition from postdoctoral fellow to junior faculty with a primary focus on prostate cancer research. In addition, experience gained through conducting this study has helped in initiating other research projects aimed at elucidating the role of telomeres in prostate cancer.

References

Cawthon, R.M. Telomere measurement by quantitative PCR. Nucleic Acids Res, May 15;30(10):e47, 2002.

Appendices